## Pending Claims

Claims 1-13 and 16 are pending.

The present invention provides a method for determining whether a test compound binds to a target RNA. The method comprises the steps of: (a) contacting the test compound with the target RNA and a RNA-modifying enzyme,which covalently alters an existing base in the target RNA; and (b) detecting the modification of the target RNA by the enzyme and comparing the amount of modification detected to that of a standard. By comparing the degree of modification, one can determine whether the test compound binds to the target RNA.

## Formal Matters

### Rejection of claim 16 under 35 U.S.C. §112, second paragraph

Claim 16 was rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Office Action states that "[t]he instant claim 16 recites 'suicide substrate' which is unclear and indefinite what the suicide substrate refers to, that is whether it refers to a specific inhibitor region or apoptotic site or enzyme suppressor region or mutant substrate region of said target RNA." The applicants respectfully traverse this rejection.

The applicants submit that the specification of the instant patent application expressly defines the term "suicide substrate." From line 29, page 13 through line 1, page 14, the specification defines the term "suicide substrate" to mean "an enzyme substrate, e.g., a target RNA or a nucleotide or base within the target RNA, that when modified by the enzyme, irreversibly binds to and inhibits the further activity of the enzyme." Thus, as will be appreciated by one of skill in the art, the suicide substrate functions as an enzyme inhibitor or inactivator by irreversibly binding to and preventing further enzymatic activity. The specification further cites Huang et al., 1998, to exemplify this term. See lines 3-6, page 14 of the specification.

In view of the above, the applicants respectfully request reconsideration and withdrawal of the rejection of claim 16 under 35 U.S.C. §112, second paragraph.

**Rejection of claims 1-11 under 35 U.S.C. §102(b)**

Claims 1-11 were rejected under 35 U.S.C. §102(b) as being anticipated by Hansen et al., "Core sequence in the RNA motif recognized by the ErmE methyltransferase revealed by relaxing the fidelity of the enzyme for its target," *RNA*, 5: 93-101 (1999).

Claim 1 was rejected on the basis that "Hansen et al. teach a method for determining whether a test compound (DMA or kethoxal) binds to a target RNA, wherein Hansen et al. discloses that the method comprises (a) contacting said test compound with said target RNA and an RNA-modifying enzyme (ErmE methyltransferase) ... that covalently alters an existing base in said target RNA ...; (b) detecting the modification of said target RNA by said enzyme and comparing the amount of modification to that of a standard (untreated control), wherein said comparison determines whether said test compound binds to said target RNA." See Office Action, penultimate paragraph, page 3. The applicants respectfully traverse this rejection.

To anticipate a claim, the reference must teach every element of the claim. Furthermore, a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. See *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987).

The applicants submit that Hansen et al. fails to teach a method for determining whether a test compound binds to a target RNA. Rather, Hansen et al. teach a correlation between the methylation fidelity in *E. coli* 23S rRNA and the magnesium concentration in the medium. Hansen et al. teach that under standard physiological conditions, the ErmE methyltransferases specifically methylate adenine at position 2058 in *E. coli* 23S rRNA. However, a reduction the magnesium concentration causes an increase in methylation of adenine sites in *E. coli* 23S rRNA by ErmE methyltransferases. The 23S rRNA structures unfold upon depletion of magnesium, making more adenine sites

accessible for ErmE methyltransferases modification. Hansen et al. further teach a second parallel experiment, whereby the chemical agents DMS and kethoxal are mixed with RNA (without enzyme) to evaluate the 23S rRNA secondary structures. DMS and kethoxal are known in the art to modify the unpaired bases on the secondary structure, and the primer extension reaction can be used to monitor the modified bases on the 23S rRNA secondary structures. Unlike Applicants' invention, the "test" compounds (DMS and Kethoxal) are never contacted with the enzyme ***(ErmE methyltransferases) in one vial.*** Thus, Hansen et al. does not teach the use of a test compound (DMS and Kethoxal) to assess its ability to (1) bind to the 23S rRNA, nor (2) inhibition of the methylation of adenine sites on the 23S rRNA by ErmE methyltransferase. Rather, DMS and kethoxal are used to **probe** the 23S rRNA secondary structure. Accordingly, neither DMA nor kethoxal are test compounds within the meaning of claim 1 in the instant application. At best, Hansen et al. can be said to teach 23S rRNA, a target RNA, and ErmE methyltransferase, a RNA modifying enzyme. Because Hansen et al. fails to teach every element of the claim either expressly or inherently, it does not anticipate the claimed invention.

Claims 2-11 were rejected on the basis that "Hansen et al. also disclose that the method comprises ribosomal RNA target ...; (ii) target RNA includes a stabilizing structure and chemical modification enhances the stability of said target RNA ...; (iii) RNA modifying enzyme is erythromycin resistance (ErmE) methyltransferase ...; (iv) target RNA modification is detected by incorporation of a radio label S-adenosyl-methionine into the target RNA ...; and (v) the test compound is a small organic molecule (DMS or kethoxal)." See paragraph bridging pages 3 and 4 of the Office Action. The applicants respectfully traverse this rejection.

The applicants submit that claims 2-11 depend from independent claim 1. For the reasons discussed above, independent claim 1, from which claims 2-11 depend, is not anticipated by Hansen et al. Thus, claims 2-11, which contain all of the limitations of claim 1, also are not anticipated by Hansen et al.

In view of the above, the applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-11 under 35 U.S.C. §102(b).

**Rejection of Claims 1-7, 9, 11 under 35 U.S.C. §102(e)**

Claims 1-7, 9, 11 were rejected on the basis that "Schwarts et al. teach a method for determining whether a test compound binds to a target nucleic acid, and that the method comprises (a) contacting said test compound with the target sample (biological fluid comprising nucleic acids) comprising RNA-modifying enzyme (S-adenosylhomocystenine hydrolase, or methyl transferases) which form s-adenosyl-L-methionine (SAM) metabolite ... that covalently alters an existing base in the target sample ...; (b) detecting the modification of said target nucleic acid by the binding of said test compound with said target RNA ..." See Office Action, lines 11-20, page 4. The applicants respectfully traverse this rejection.

To anticipate a claim, the reference must teach every element of the claim. Furthermore, a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. See *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987).

The applicants submit that Schwarts et al. fail to teach "the binding of said test compound with said target RNA." Rather, Schwarts et al. teach: (1) a SAM-mediated methyl transferase (a target modifying enzyme) that methylates a target substrate such as RNA (target RNA), whereas s-adenosylhomocysteine (SAH), a product of the SAM-mediated methylation process, acts as a differential inhibitor of SAM-mediated methylation. In addition, Schwarts et al. teach a method of evaluating the impact of a test compound on a target cellular process characteristic of a disease or condition (see col. 5, line 64 through col. 6, line39). The examples of target processes include transport of molecules across the cell membrane, intracellular localization of molecules in organelles or compartments, intercellular and intracellular signalling pathways, metabolic processes, and the like (see col. 6, lines 12-16 of Schwarts et al.). One of ordinary skill in the art

would recognize that Schwarts et al., at most, teach a RNA modifying enzyme (methyl transferase), a target RNA, and a test compound (SAH) that inhibits the RNA modifying enzyme. However, Schwarts et al. fails to expressly teach that SAH inhibits the SAM-mediated methyl transferase via binding to the target RNA.

Nor does Schwartz et al. inherently teach, "binding of SAH to the substrate RNA." The Federal Circuit has held that "[a] reference includes an inherent characteristic if that characteristic is the 'natural result' flowing from the reference's explicitly explicated limitations." See *Continental Can Co. USA, INC. v Monsanto Co.*, 948 F.2d 1264 (Fed. Cir. 1991). Furthermore, the fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) . . . ; *In re Oelrich*, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981). "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.' " See *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999). Based on Schwarts et al., one of ordinary skill in the art would recognize that "the binding of SAH (the "compound")to the substrate RNA" is neither a "necessary" nor a "natural result" flowing from the Schwarts et al. disclosure. To the contrary, Yi, P. et al. teaches against inherency, since this reference teaches that SAH inhibits the SAM-mediated methyl transferase by binding to the enzyme (see Yi, P. et al., "Increase in plasma homocysteine associated with parallel increases in plasma S-Adenosylhomocysteine and lymphocyte DNA hypomethylation," *J. Bio. Chem.*, 275(38): 29318 – 29323 (2002). Therefore, Schwarts et al. cannot inherently teach the binding of SAH to the methyl transferase.

In view of the above, the applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-7, 9, and 11 under 35 U.S.C. §102(b).

**Rejection of Claims 1-4 and 16 under 35 U.S.C. §102(b)**

Claims 1-4 and 16 were rejected on the basis that "Glazer et al. teach a method for determining a test compound (neplanocin A, a.k.a. NPC) binds to a target RNA, wherein Glazer et al. teach that the method comprises (a) contacting a test compound with a RNA-modifying enzyme (RNA methyltransferase) and said target RNA comprising "suicide substrate" (cytocidal substrate) for said enzyme (see page 12964, colum 1, summary, column, paragraphs 1-8); (b) detecting the modification of the enzyme by said suicide substrate (decreased RNA methylation), wherein said detecting determines whether said test compound binds to said target RNA (see page 12965, column 1, paragraphs 1-5, column 2, paragraph 1-2)." The applicants respectfully traverse this rejection.

To anticipate a claim, as discussed above, the reference must teach every element of the claim. Furthermore, a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. See *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987).

The applicants submit that Glazer et al. fails to teach every element of the claims, Rather, Glazer et al. expressly teaches that neplanocin A (hereinafter "NPC") inhibits RNA methylation, and that NPC does not inhibit rRNA synthesis. Namely that NPC inhibits RNA methylation via binding to the substrate RNA.

Nor does, Glazer et al. inherently teach the binding of NPC to the substrate RNA. Briefly, as discussed above, "[a] reference includes an inherent characteristic if that characteristic is the 'natural result' flowing from the reference's explicitly explicated limitations." See *Continental Can Co. USA, INC. v Monsanto Co.*, 948 F.2d 1264 (Fed. Cir. 1991). "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.' " See *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999).

The applicants submit that NPC inhibits methylation through binding to S-adenosylhomocysteine hydrolase, an enzyme involved in **regulating** methytransferase activity. Thus, rather than binding to "a target RNA", as required by independent claims 1 and 16, the Blazer et al. reference teaches binding of a "test" compound (NPC) to a regulatory (non-modifying) component. In Borchardt, RT et al., "Neplanocin A, a potent inhibitor of S-adenosylhomocysteine hydrolase and of vaccinia virus," *J. Bio. Chem.*, 259(7): 4353-4358 (1984), Neplanocin A (NPC) is shown to inhibit S-adenosylhomocysteine hydrolase (SAAH) by binding to SAAH, and the binding is related to inhibition of S-adenosylmethionine(SAM)-dependent methylation of RNA. Furthermore, enzyme SAAH is known in the art to promote enzyme SAAH promotes methylation by cleaving a byproduct of all S-adenosymethionine (SAM)-dependent methylation reactions, whereas the byproduct inhibits the methylation reactions by binding to SAM-mediated methyltranferases, and that the inhibition of the SAAH enzymatic activity by NPC serves to inhibit the methylation reactions. See Kramer, DL et al., "Combined modulation of S-adenosylmethionine biosynthesis and S-adenosylhomocysteine metabolism enhances inhibition of nucleic acid methylation and L 1210 cell growth," *Cancer Res.*, 50: 3838-3842 (1990). Thus, NPC ("test" compound) does not inhibit methylation by binding to the target RNA, as required by Applicants' claims, rather, it inhibits methylation by binding to SAAH, a <u>non</u>-RNA modifying enzyme that promotes methyltransferase activity by cleaving an inhibiting byproduct of methylation reactions. Based on this discussion, the Applicants respectfully submit that Glazer et al. fails to/cannot teach a test compound that inhibits the RNA modifying enzyme via binding to the RNA substrate, either expressly or inherently.

In view of the above, the applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-4 and 16 under 35 U.S.C. §102(b).

**<u>Rejection of Claims 12 and 13 under 35 U.S.C. §103(a)</u>**

Claims 12 and 13 were rejected under 35 U.S.C. §103(a) as being unpatentable over Hansen et al. in view of Karn et al. (U.S. Pat. No. 6,316,194). The Office Action states that Hansen et al. teach a method for determining whether a test compound (DMA

or Kethoxal) binds to a target RNA. See Office Action line 8, page 6 through line 2, page 7. The Office Action further states that Karn et al. teach a method for determining whether a test compound binds to a target RNA comprising incubating a test compound with the target RNA and an antimicrobial molecule, measuring or detecting the change or modification of said target RNA, and comparing the amount of the change to that of a standard to identify the test compound that binds to the target RNA. The Office Action also states that the method by Karn et al. is designed for a high-throughput screening format. See Office Action lines 3-16, page 7. The applicants respectfully traverse this rejection.

The applicants submit that the examiner has failed to establish a *prima facie* case of obviousness under 35 U.S.C. §103(a). As discussed above, rather than teaching a method for determining whether a test compound binds to a target RNA, Hansen et al. teach a relationship between the methylation fidelity in *E. coli* 23S rRNA and the magnesium concentration in the medium. According to Hansen et al., as the magnesium concentration decreases, more adenine sites in *E. coli* 23S rRNA are methylated by ErmE methyltransferases. The 23S rRNA structures unfold on depletion of magnesium, making more adenine sites accessible for ErmE methyltransferases modification. In contrast, the assays of the present invention are designed to identify compounds that inhibit enzymatic modifications of RNA which result in antibiotic resistance and RNA maturation. See lines 6-8, page 1 of the specification of the instant application. To achieve this objective, the present invention provides a method for determining whether a test compound binds to a target RNA, the method comprising the steps of: (a) contacting the test compound with the target RNA and a RNA-modifying enzyme that covalently alters an existing base in said target RNA; and (b) detecting the modification of the target RNA by the enzyme and comparing the amount of modification detected to that of a standard, wherein the comparing determines whether the test compound binds to the target RNA. Therefore, one of ordinary skill in the art would not have been motivated at the time of the applicants' invention to combine the primary reference, Hansen et al., with the secondary reference, Karn et al, to design the claimed method in the instant application.

Moreover, the purpose of the Hansen et al. reference was to elucidate the mechanism by which variations in the magnesium concentration affects RNA structure and ErmE methyltransferase activity. Thus, Hansen et al. do not recognize the source of the problem (enzymatic modifications of RNA that result in antibiotic resistance and RNA mutation) solved by the claimed invention. In *In re Sponnoble*, 405 F.2d 578 (CCPA 1969), the court held that because the prior art did not recognize the cause of the problem intended to be solved by the invention in the patent application, the invention was not obvious over the prior art. See *Id.* at 586-587 ("The question here is whether the prior art recognized the cause of the problem. ... That a natural rubber plug might be permeable to steam in no way establishes or makes obvious its permeability to liquid water; thus the cause of the problem is not suggested by the prior art.").

For the reasons given above, the applicants respectfully submit that the examiner has failed to establish a *prima facie* case of obviousness of claims 12 and 13 under 35 U.S.C. §103(a), and request reconsideration and withdrawal of the rejection.

## Conclusions

The applicants submit that all grounds of the rejections have been properly traversed and all claims are allowable as written, and respectfully request prompt and favorable action by the examiner. If the examiner believes that a telephone conversation with Applicants' attorney would expedite prosecution of this application, the examiner is cordially invited to call the undersigned attorney of record.

Respectfully submitted,

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